Preparing Cells Competent for Recombineering

Overview The preparation of recombineering-proficient cells that are ready for electrotransformation

Duration About 3 hours

Preparation The previous day, grow a 5 ml overnight culture of the chosen recombineering cells at 30-32°C. Include the appropriate drug if a plasmid is supplying the Red functions.

Caution *Do not grow recombineering cells at temperatures greater than 34°C.*

Caution *Maintain sterile technique throughout the rest of the protocol.*

1 Dilute the overnight culture by adding 0.5 ml of the overnight to 35 ml of LB medium with the appropriate drug(s) if needed, in a 250 ml (or 125 ml) baffled Erlenmeyer flask. Dilute the overnight at least 70-fold. Grow cells in a H_2O bath at 32°C with shaking (200rpm) until the OD_{600} is from 0.4-0.5 (approximately 2 hrs).

Tip *Cells with different genotypes will grow at different rates. Having the proper OD_{600} is critical – the recombination will not work if the density is too high.*

Tip *Only add drug to the LB if it is needed to maintain a plasmid.*

2 Transfer half the culture to a 50 ml baffled Erlenmeyer flask and place that flask in a 42ºC H_2O bath to shake at 200rpm; keep the other flask at 32ºC. Shake for 15 min. The culture at 42ºC is now induced for the recombination functions and the 32ºC culture is the uninduced control. Both flasks will be processed identically during the rest of the protocol.

Tip *If you do not have baffled flasks, use a 125 ml or larger flask.*

3 Immediately after the 15 min induction, rapidly chill both cultures in an ice-water slurry; swirl the flasks gently. Leave on ice for 5-10 min. Label and chill the necessary number of 35-50 ml centrifuge tubes for the induced and uninduced cells.

Tip *Pre-chill the sterile distilled H_2O that will be used for washes. Keep 200 ml bottles of distilled water at 4°C for this purpose and put it on ice as needed. Also chill electrotransformation cuvettes and microcentrifuge tubes for later parts of this step.*

4 Transfer both the induced and uninduced cultures to the chilled centrifuge tubes and centrifuge 7 min at ~6500 x *g* (6700 rpm in a Sorvall SA-600 rotor) at 4ºC. Using sterile technique, aspirate or pour off supernatant.

5 Add 1 ml ice-cold sterile distilled H_2O to the cell pellet and gently suspend cells with a large disposable pipet tip (do not vortex). After cells are well suspended, add another 30 ml of ice-cold distilled H_2O to each tube, seal, and gently invert to mix, again without vortexing. Centrifuge tubes again as in previous step.

6 Promptly decant the 30 ml supernatant very carefully from the soft pellet in each tube and gently suspend each cell pellet in 1 ml ice-cold distilled H_2O.

Tip *As the pellets are very soft, tubes must be removed promptly after centrifugation and care should be taken not to dislodge the pellet. It is ok at this step to leave a small amount of supernatant in the tube.*

7 Transfer the suspended cells to pre-chilled microcentrifuge tubes. Centrifuge 30 sec at maximum speed in a 4°C refrigerated microcentrifuge. Carefully remove the supernatant and suspend cells in 200 µl sterile ice-cold distilled H_2O and keep on ice until used.

Tip *This protocol will prepare enough cells for four or five electroporations. If more cells are needed, the best way to do so is to prepare additional flasks.*